

CLAIMS

1. A method of producing (S)-2-pentanol which comprises allowing microorganisms or transformed cells, a product obtained by treating said microorganisms or cells, a culture solution of said microorganisms or cells, and/or a crude purified product or purified product of a carbonyl reductase fraction obtained from said microorganisms or cells, to act on 2-pentanone, wherein when a fresh cell mass of said microorganisms or transformed cells, which has not been pretreated with a solvent, is allowed to act on 2-pentanone, (S)-2-pentanol having an optical purity of 95% e.e. or greater can be generated, and the productivity thereof is 1 mg or more of (S)-2-pentanol/g of dry cell mass weight/hour.

2. A method of producing (S)-2-hexanol which comprises allowing microorganisms or transformed cells, a product obtained by treating said microorganisms or cells, a culture solution of said microorganisms or cells, and/or a crude purified product or purified product of a carbonyl reductase fraction obtained from said microorganisms or cells, to act on 2-hexanone, wherein when a fresh cell mass of said microorganisms or transformed cells, which has not been pretreated with a solvent, is allowed to act on 2-hexanone, (S)-2-hexanol having an optical purity of 95% e.e. or greater can be generated, and the productivity thereof is 1 mg or more of (S)-2-hexanol/g of dry cell mass weight/hour.

3. A method for producing (S)-2-pentanol or (S)-2-hexanol having high optical purity, wherein microorganisms selected from the group consisting of genus *Brettanomyces*, genus *Candida*, genus *Hortaea*, genus *Issatchenkia*, genus *Lodderomyces*, genus *Pichia*, genus *Rhodotorula*, genus *Arthrobacter*, genus *Brevibacterium*, genus *Crutobacterium*, genus *Geobacillus*, genus *Microbacterium*, genus *Ochrobactrum*, genus *Paracoccus*, genus *Rhizobium*, and genus *Rhodococcus*, a product obtained by treating said microorganisms, a culture solution of said microorganisms, and/or a crude purified

product or purified product of a carbonyl reductase fraction obtained from said microorganisms, are allowed to act on 2-pentanone or 2-hexanone, so as to generate (S)-2-pentanol or (S)-2-hexanol.

4. A method for producing (S)-2-pentanol or (S)-2-hexanol having high optical purity, wherein transformed cells wherein DNA encoding carbonyl reductase obtained from microorganisms selected from the group consisting of genus *Brettanomyces*, genus *Candida*, genus *Hortaea*, genus *Issatchenkia*, genus *Lodderomyces*, genus *Pichia*, genus *Rhodotorula*, genus *Arthrobacter*, genus *Brevibacterium*, genus *Crutobacterium*, genus *Geobacillus*, genus *Microbacterium*, genus *Ochrobactrum*, genus *Paracoccus*, genus *Rhizobium*, and genus *Rhodococcus*, has been allowed to express, a product obtained by treating said cells, a culture solution of said cells, and/or a crude purified product or purified product of a carbonyl reductase fraction obtained from said cells, are allowed to act on 2-pentanone or 2-hexanone, so as to generate (S)-2-pentanol or (S)-2-hexanol.

5. The production method according to claim 3 or 4, wherein the microorganisms are selected from the group consisting of *Brettanomyces bruxellensis*, *Brettanomyces anomalus*, *Candida famata*, *Candida krusei*, *Candida maltosa*, *Candida tropicalis*, *Candida zeylanoides*, *Hortaea werneckii*, *Issatchenkia scutulata*, *Lodderomyces elongisporus*, *Pichia angusta*, *Pichia besseyi*, *Pichia cactophila*, *Pichia segobiensis*, *Pichia spartinae*, *Pichia trehalophila*, *Rhodotorula minuta*, *Arthrobacter oxydans*, *Arthrobacter polychromogenes*, *Arthrobacter* sp., *Arthrobacter sulfurous*, *Brevibacterium butanicum*, *Curtobacterium flaccumfaciens*, *Geobacillus stearothermophilus*, *Microbacterium keratanolyticum*, *Microbacterium saperdae*, *Microbacterium* sp., *Microbacterium testaceum*, *Ochrobactrum anthropi*, *Ochrobactrum* sp. (*Pseudomonas ovalis*), *Pracoccus denitrificans*, *Rhizobium radiobacter*, and *Rhodococcus* sp. (*Corynebacterium hydrocarboclastum*).

6. A method for producing (S)-2-pentanol or (S)-2-hexanol having high optical purity, wherein transformed cells, wherein DNA described in any one of the following

(A) to (F) has been allowed to express, a product obtained by treating said cells, and/or a culture solution of said cells, are allowed to act on 2-pentanone or 2-hexanone, so as to generate (S)-2-pentanol or (S)-2-hexanol:

(A) DNA encoding a protein having the amino acid sequence shown in SEQ ID NO: 1;

(B) DNA encoding a protein, which has an amino acid sequence comprising a deletion, addition, or substitution of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and which has ability to reduce a carbonyl group to synthesize optically active alcohol;

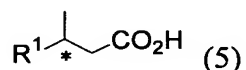
(C) DNA encoding a protein, which has an amino acid sequence showing homology of 50% or more with the amino acid sequence shown in SEQ ID NO: 1, and which has ability to reduce a carbonyl group to synthesize optically active alcohol;

(D) DNA having the nucleotide sequence shown in SEQ ID NO: 2;

(E) DNA having a nucleotide sequence, which comprises a deletion, addition, or substitution of one or several nucleotides with respect to the nucleotide sequence shown in SEQ ID NO: 2, and which encodes a protein having ability to reduce a carbonyl group to synthesize optically active alcohol; and

(F) DNA having a nucleotide sequence, which hybridizes with the nucleotide sequence shown in SEQ ID NO: 2 or a complementary sequence thereof under stringent conditions, and which encodes a protein having ability to reduce a carbonyl group to synthesize optically active alcohol.

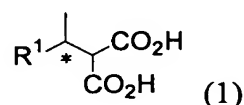
7. A method for producing (R)- or (S)-3-methyl carboxylic acid represented by the following formula (5):



wherein R¹ represents an alkyl group containing 3 to 5 carbon atoms, and * represents an asymmetric carbon,

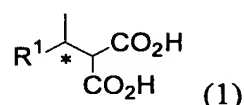
which comprises decarboxylating (R)- or (S)-1-methylalkyl malonic acid having

optical activity represented by the following formula (1) in the presence of a highly polar solvent and/or an additive for promoting decarboxylation:



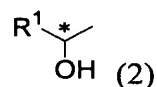
wherein R¹ has the same definition as described above, and * represents an asymmetric carbon.

8. A method for producing (R)- or (S)-1-methylalkyl malonic acid represented by the following formula (1):

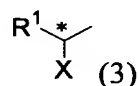


wherein R¹ represents an alkyl group containing 3 to 5 carbon atoms, and * represents an asymmetric carbon,

which comprises allowing optically active alcohol represented by the following formula (2) to react with a sulfonylation agent:

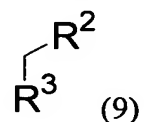


wherein R¹ has the same definition as described above, and * represents an asymmetric carbon, so as to obtain an optically active compound represented by the following formula (3):

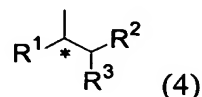


wherein R¹ has the same definition as described above, X represents a sulfonyloxy group, and * represents an asymmetric carbon;

allowing the optically active compound to react with a carbon nucleophile represented by the following formula (9) in the presence of a base:



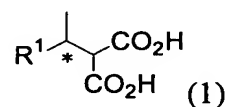
wherein each of R² and R³ independently represents an ester group, a carboxyl group, or a cyano group, wherein R² and R³ may together form a cyclic structure, so as to obtain an optically active compound represented by the following formula (4):



wherein R¹, R², and R³ have the same definitions as described above, and * represents an asymmetric carbon, and

hydrolyzing the obtained optically active compound.

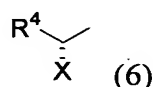
9. (R)-1-methylalkyl malonic acid or (S)-1-methylalkyl malonic acid having an optical purity of 90%ee or greater, which is represented by the following formula (1):



wherein R¹ represents an alkyl group containing 3 to 5 carbon atoms, and * represents an asymmetric carbon.

10. The (R)-1-methylalkyl malonic acid or (S)-1-methylalkyl malonic acid according to claim 9, wherein R¹ represents an n-propyl group or an n-butyl group.

11. A method for producing an optically active substance represented by the following formula (6):

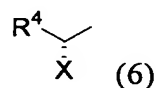


wherein R⁴ represents an n-propyl group, and X represents a sulfonyloxy group,

which comprises: allowing microorganisms or transformed cells containing a carbonyl reductase having activity to react with 2-pentanone to generate (S)-2-pentanol, wherein it is able to generate (S)-2-pentanol having an optical purity of 95% e.e. or greater when the fresh cell mass thereof, which has not been pretreated with a solvent, is allowed to act on 2-pentanone, and the productivity thereof is 10 mg or more of (S)-2-pentanol/g of dry cell mass weight/hour, a product obtained by treating said

microorganisms or cells, a culture solution of said microorganisms or cells, and/or a crude purified product or purified product of a carbonyl reductase fraction obtained from said microorganisms or cells, to act on 2-pentanone, so as to convert it to (S)-2-pentanol; and allowing the obtained (S)-2-pentanol to react with a sulfonylation agent, so as to convert it to the optically active substance represented by the above formula (6).

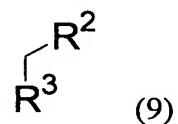
12. A method for producing an optically active substance represented by the following formula (6):



wherein R⁴ represents an n-butyl group, and X represents a sulfonyloxy group,

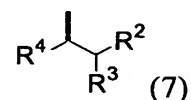
which comprises: allowing microorganisms or transformed cells containing a carbonyl reductase having activity to react with 2-hexanone to generate (S)-2-hexanol, wherein it is able to generate (S)-2-hexanol having an optical purity of 95% e.e. or greater when the fresh cell mass thereof, which has not been pretreated with a solvent, is allowed to act on 2-hexanone, and the productivity thereof is 10 mg or more of (S)-2-hexanol/g of dry cell mass weight/hour, a product obtained by treating said microorganisms or cells, a culture solution of said microorganisms or cells, and/or a crude purified product or purified product of a carbonyl reductase fraction obtained from said microorganisms or cells, to act on 2-hexanone, so as to convert it to (S)-2-hexanol; and allowing the obtained (S)-2-hexanol to react with a sulfonylation agent, so as to convert it to the optically active substance represented by the above formula (6).

13. The method according to claim 11 or 12, which further comprises a step of allowing the optically active substance represented by formula (6) to react with a carbon nucleophile represented by the following formula (9) in the presence of a base:



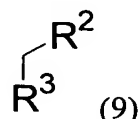
wherein each of R² and R³ independently represents an ester group, a carboxyl group, or

a cyano group, wherein R² and R³ may together form a cyclic structure, so as to convert it to an optically active compound represented by the following formula (7):

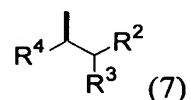


wherein R² and R³ have the same definitions as described above, and R⁴ represents an n-propyl group or an n-butyl group.

14. A method for producing (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid, which comprises; allowing the optically active substance represented by formula (6) obtained by the method according to claim 11 or 12 to react with a carbon nucleophile represented by the following formula (9) in the presence of a base:

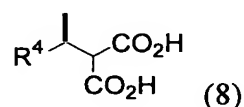


wherein each of R² and R³ independently represents an ester group, a carboxyl group, or a cyano group, wherein R² and R³ may together form a cyclic structure, so as to convert it to an optically active compound represented by the following formula (7):



wherein R² and R³ have the same definitions as described above, and R⁴ represents an n-propyl group or an n-butyl group, and

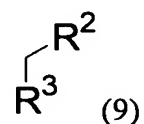
hydrolyzing the obtained optically active compound, so as to convert it to (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid represented by the following formula (8):



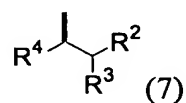
wherein R⁴ has the same definition as described above.

15. A method for producing (R)-3-methyl hexanoic acid or (R)-3-methyl heptanoic acid, which comprises allowing the optically active substance represented by formula (6)

obtained by the method according to claim 11 or 12 to react with a carbon nucleophile represented by the following formula (9) in the presence of a base:

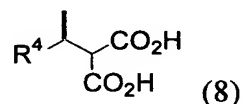


wherein each of R^2 and R^3 independently represents an ester group, a carboxyl group, or a cyano group, wherein R^2 and R^3 may together form a cyclic structure, so as to convert it to an optically active compound represented by the following formula (7):



wherein R^2 and R^3 have the same definitions as described above, and R^4 represents an n-propyl group or an n-butyl group,

hydrolyzing the obtained optically active compound, so as to convert it to (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid represented by the following formula (8):



wherein R^4 has the same definition as described above, and

decarboxylating the obtained (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid.